

WHAT IS CLAIMED IS:

- 1 1. A method of evolving a biocatalytic
2 activity of a cell, comprising:
3 (a) recombining at least a first and second
4 DNA segment from at least one gene conferring ability to
5 catalyze a reaction of interest, the segments differing
6 from each other in at least two nucleotides, to produce a
7 library of recombinant genes;
8 (b) screening at least one recombinant gene
9 from the library that confers enhanced ability to
10 catalyze the reaction of interest by the cell relative to
11 a wildtype form of the gene;
12 (c) recombining at least a segment from the at
13 least one recombinant gene with a further DNA segment
14 from the at least one gene, the same or different from
15 the first and second segments, to produce a further
16 library of recombinant genes;
17 (d) screening at least one further recombinant
18 gene from the further library of recombinant genes that
19 confers enhanced ability to catalyze the reaction of
20 interest by the cell relative to a previous recombinant
21 gene;
22 (e) repeating (c) and (d), as necessary, until
23 the further recombinant gene confers a desired level of
24 enhanced ability to catalyze the reaction of interest by
25 the cell.

1 2. The method of claim 1, wherein the
2 reaction of interest is the ability to utilize a
3 substrate as a nutrient source.

1 3. The method of claim 1, wherein the
2 reaction of interest is the ability to catabolize a
3 compound.

1 4. The method of claim 1, wherein the
2 reaction of interest is the ability to detoxify a
3 compound.

1 5. The method of claim 1, wherein the
2 reaction of interest is the ability to synthesize a
3 compound of interest.

1 6. The method of claim 4, wherein the
2 compound is an antibiotic.

1 7. The method of claim 4, wherein the
2 compound is an amino acid.

1 8. The method of claim 4, wherein the
2 compound is a polymer.

1 9. The method of claim 4, wherein the
2 compound is a carotenoid.

1 10. The method of claim 4, wherein the
2 compound is vitamin C.

1 11. The method of claim 4, wherein the
2 compound is indigo.

1 12. The method of claim 1, wherein at least
2 one recombining step is performed *in vitro*, and the
3 resulting library of recombinants is introduced into the
4 cell whose biocatalytic activity is to be enhanced
5 generating a library of cells containing different
6 recombinants.

1 13. The method of claim 12, wherein the *in*
2 *vitro* recombining step comprises:

3 cleaving the first and second segments into
4 fragments;

1 mixing and denaturing the fragments; and
2 incubating the denatured fragments with a
3 polymerase under conditions which result in annealing of
4 the denatured fragments and formation of the library of
5 recombinant genes.

1 14. The method of claim 1, wherein at least
2 one recombining step is performed *in vivo*.

1 15. The method of claim 1, wherein the
2 recombining step is performed in the cell whose
3 biocatalytic activity is to be enhanced.

1 16. The method of claim 1, wherein at least
2 one DNA segment comprises a cluster of genes collectively
3 conferring ability to catalyze a reaction of interest.

1 17. A method of evolving a gene to confer
2 ability to catalyze a reaction of interest, the method
3 comprising:

4 (1) recombining at least first and second DNA
5 segments from at least one gene conferring ability to
6 catalyze a reaction of interest, the segments differing
7 from each other in at least two nucleotides, to produce a
8 library of recombinant genes;

9 (2) screening at least one recombinant gene
10 from the library that confers enhanced ability to
11 catalyze a reaction of interest relative to a wildtype
12 form of the gene;

13 (3) recombining at least a segment from the at
14 least one recombinant gene with a further DNA segment
15 from the at least one gene, the same or different from
16 the first and second segments, to produce a further
17 library of recombinant genes;

18 (4) screening at least one further recombinant
19 gene from the further library of recombinant genes that

20 confers enhanced ability to catalyze a reaction of
21 interest relative to a previous recombinant gene;
22 (5) repeating (3) and (4), as necessary, until
23 the further recombinant gene confers a desired level of
24 enhanced ability to catalyze a reaction of interest.

1 18. A method of generating a new biocatalytic
2 activity in a cell, comprising:

3 (1) recombining at least first and second DNA
4 segments from at least one gene conferring ability to
5 catalyze a first reaction related to a second reaction of
6 interest, the segments differing from each other in at
7 least two nucleotides, to produce a library of
8 recombinant genes;

9 (2) screening at least one recombinant gene
10 from the library that confers a new ability to catalyze
11 the second reaction of interest;

12 (3) recombining at least a segment from at
13 least one recombinant gene with a further DNA segment
14 from the at least one gene, the same or different from
15 the first and second segments, to produce a further
16 library of recombinant genes;

17 (4) screening at least one further recombinant
18 gene from the further library of recombinant genes that
19 confers enhanced ability to catalyze the second reaction
20 of interest in the cell relative to a previous
21 recombinant gene;

22 (5) repeating (3) and (4), as necessary, until
23 the further recombinant gene confers a desired level of
24 enhanced ability to catalyze the second reaction of
25 interest in the cell.

1 19. A modified form of a cell, wherein the
2 modification comprises a metabolic pathway evolved by
3 recursive sequence recombination.

1 20. A method of optimizing expression of a
2 gene product, the method comprising:

3 (1) recombining at least first and second DNA
4 segments from at least one gene conferring ability to
5 produce the gene product, the segments differing from
6 each other in at least two nucleotides, to produce a
7 library of recombinant genes;

8 (2) screening at least one recombinant gene
9 from the library that confers optimized expression of the
10 gene product relative to a wildtype form of the gene;

11 (3) recombining at least a segment from the at
12 least one recombinant gene with a further DNA segment
13 from the at least one gene, the same or different from
14 the first and second segments, to produce a further
15 library of recombinant genes;

16 (4) screening at least one further recombinant
17 gene from the further library of recombinant genes that
18 confers optimized ability to produce the gene product
19 relative to a previous recombinant gene;

20 (5) repeating (3) and (4), as necessary, until
21 the further recombinant gene confers a desired level of
22 optimized ability to express the gene product.

1 21. The method of claim 20, wherein the at
2 least one gene encodes the gene product.

1 22. The method of claim 20, wherein the at
2 least one gene is a vector comprising a gene encoding the
3 gene product.

1 23. The method of claim 20, wherein at least
2 one recombining step is performed *in vivo*.

1 24. The method of claim 23, wherein the
2 recombining step is performed in a host cell wherein the
3 gene product is expressed.

1 25. The method of claim 20, wherein the at
2 least one gene is a host cell gene and wherein the host
3 cell gene does not encode the gene product.

1 26. The method of claim 20, wherein
2 optimization results in increased expression of the gene
3 product.

1 27. A method of evolving a biosensor for a
2 compound A of interest, the method comprising:

3 (1) recombining at least first and second DNA
4 segments from at least one gene conferring ability to
5 detect a related compound B, the segments differing from
6 each other in at least two nucleotides, to produce a
7 library of recombinant genes;

8 (2) screening at least one recombinant gene
9 from the library that confers optimized ability to detect
10 compound A relative to a wildtype form of the gene;

11 (3) recombining at least a segment from the at
12 least one recombinant gene with a further DNA segment
13 from the at least one gene, the same or different from
14 the first and second segments, to produce a further
15 library of recombinant genes;

16 (4) screening at least one further recombinant
17 gene from the further library of recombinant genes that
18 confers optimized ability to detect compound A relative
19 to a previous recombinant gene;

20 (5) repeating (3) and (4), as necessary, until
21 the further recombinant gene confers a desired level of
22 optimized ability to detect compound A.

1 28. The method of claim 27, wherein
2 optimization results in increased amplitude of response
3 by the biosensor.

1 29. The method of claim 27, wherein compound A
2 and compound B are different.

- 1 30. The method of claim 27, wherein compound A
2 and compound B are identical.

INS
DR